Deterrent Effects of Four Neem-Based Formulations on Gravid Female Boll Weevil (Coleoptera: Curculionidae) Feeding and Oviposition on Cotton Squares

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ABSTRACT Three commercial neem-based insecticides, Agroneem, Ecozin, and Neemix, and a neem seed extract formulation, bitters, containing 1,036, 16,506, 471, and 223 μg/ml azadirachtin, respectively, were assessed for feeding and oviposition deterrence against gravid female boll weevils, *Anthonomus grandis grandis* Boheman, in the laboratory. In choice assays, excised cotton squares dipped in the separate formulations were first physically contacted by the weevils' tarsi or antennae fewer times than nontreated control squares. In choice and no-choice assays, each formulation repelled the weevils for ≥90 min. After 24 h in the choice assays, feeding punctures on the squares treated with Agroneem, Ecozin, or bitters were significantly fewer compared with controls. Egg punctures on the Ecozin- and the bitters-treated squares were significantly fewer than on control squares after 24 h. In the no-choice assay, no significant difference was detected. Aging the formulations under outdoor conditions for 24 h before weevils were exposed resulted in 46–60% and 62–82% reductions in feeding and oviposition punctures, respectively, compared with controls. Agroneem- and bitters-treated squares had >37% fewer feeding punctures after being aged for 48 h. No significant difference was detected after 72 h of aging. Because the deterrence of the gravid female boll weevils was not correlated with amounts of azadirachtin, azadirachtin does not seem to be the only, or the most influential, component of neem that induced the observed deterrence.

KEY WORDS *Anthonomus grandis*, azadirachtin, boll weevil, cotton, neem

DURING THE COTTON, *Gossypium hirsutum* L., growing season, most commercial growers rely on conventional insecticides to protect against crop losses caused by the boll weevil, *Anthonomus grandis grandis* Boheman, (Loera-Gallardo et al. 1997, Page et al. 1999). Predators (Sterling 1978, Sturm et al. 1990), parasitoids (Morales-Ramos and King 1991; Summy et al. 1997a,b), trap crops (Moore and Watson 1990), and kaolin particle film (Showler 2002) have been reported as being effective in experimental conditions.

Botanical compounds represent an alternative to conventional pesticides and extracts of creeping oxeye, *Wedelia biflora* Jacq., and Chinese water chestnut, *Eleocharis dulcis* Trin., for example, are known to have antifeedant effects on boll weevils. The neem tree, *Azadirachta indica* A. Juss (Meliaceae), has insecticidal properties (Koul et al. 1990). A major active constituent in neem extracts is the tetranortriterpenoid limonoid, azadirachtin, known for deterrent, antifeedant, toxic, and growth regulator effects (Schmutterer 1990, Mordue and Blackwell 1993). Other insecticidal compounds found in neem extracts include salannin, salannol, salannolacetate, nimbinen, gedunin, dirachtin, and viselinin derivatives (Jones et al. 1989, Walter 1999). Research on curculionids has been sparse. Neem extracts are repellent to rice weevils, *Sitophilus oryzae* (L.), whereas neem seed powder mixed with wheat, *Triticum aestivum* L. Pers., kernels provided 9–12 mo of protection against rice weevil, *Oryzae sativa* L., in stored rice (Jotwani and Sircar 1965). Populations of rice weevils were reduced when exposed to wheat kernels treated with a commercial neem-based insecticide (Dunkel et al. 1990). Pea and bean weevil, *Sitona lineatus* L., damage to field beans, *Phaseolus* spp., declined in plots sprayed with a 50% neem formulation (Smart et al. 1994). White pine weevils, *Pissodes strobi* Peck, however, were not affected by the delivery of neem seed extract to two spruce (*Picea*) species (Naumann et al. 1996). In this study, we assess deterrent effects of commercial and custom-formulated neem-based insecticides, with different azadirachtin content, on the boll weevil, under laboratory conditions.

Materials and Methods

Assays were conducted at the USDA–ARS Kika de la Garza Subtropical Agricultural Research Center (SARC) in Weslaco, Hidalgo County, TX. The cotton variety used was C-208 (UAP Southwest, Santa Rosa,

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TX), grown in 7.5-liter pots, each with three plants. Reference to "7-mm square diameter stage" cotton plants means that at least five squares (flower buds) on the plants were 7 mm in diameter before blooming began. The boll weevils used in this study were collected from Hercon (Hercon Environmental, Emigsville, PA) traps with grandlure strips in Hidalgo County. Captured weevils were sustained in the laboratory on fresh cotton squares in 30-cm^3 cages until they were used in the assays. Gravid female boll weevils were obtained as described by Showler (2002).

Liquid formulations of three commercial neem-based insecticides, Agroneem (Ajay Bio-Tech, Pune, India), Ecozin (AmVaC, Los Angeles, CA), and Neemix 4.5 (Certis, Columbia, MD), were used. A noncommercial neem seed extract, bitters, that was collected by a patented process (U.S. Patent Office, 2001) had an azadirachtin content of 223 μg/ml. Determination of azadirachtin content involved dilution with ethanol to ensure solubility in the high-performance liquid chromatography (HPLC) mobile phase. Neemix and Agroneem samples were diluted 1:1 (vol:vol). Ecozin and bitters were diluted 1:2 to quantitate the Ecozin (at higher dosages, the azadirachtin peak was above the limit of linear absorption for the detector) and because of the greater viscosity of the bitters. The HPLC analysis method was adapted from Carboni et al. (2002) for use on a Hewlett-Packard (Palo Alto, CA) LC Series 1100. The solvent system was comprised of water (mobile phase A) and acetonitrile (mobile phase B). Starting conditions were 85% A and 15% B. The gradient was 15–45% B in 2 min; 45–100% B in 13 min; hold 100% for 2 min, 100–15% B in 2 min. The column was allowed to reequilibrate for 6 min between injections. A C8 3.5-μm column, 150 by 4.6 mm, was used in a 50°C column oven to achieve compound separation. The flow rate was 1 ml/min, and detection was at 215 nm. Five microliters of each sample was injected. Azadirachtin eluted at 5.73 min.

Concentrations of azadirachtin in the Agroneem, Ecozin, and Neemix were 1,036, 16,506, and 471 μg/ml, respectively, before dilution. Agroneem, Ecozin, and Neemix were mixed, in accordance with the manufacturers' labels, with distilled water to 0.81 (vol:vol), 0.2, and 2.0%, respectively. The bitters was formulated for application by combining 1 ml of the bitters with 200 ml of distilled water and 20 ml each of methyl alcohol and surfactant (Silwet L-77, Helena Chemical Corporation, Memphis, TN). All of the neem formulations were used in the bioassays in this study on the same day they were diluted.

First Contact with Treated or Nontreated Excised Squares. One gravid female boll weevil was released in a 14.5-cm-diameter petri dish for 5 min. One excised, debracted 7-mm-diameter cotton square was dipped in one of the neem formulations, allowed to dry for 20 min, and placed in the petri dish 10 cm apart from a debracted nontreated control. Both squares were placed equidistant from the boll weevil. The square that was contacted first by the boll weevil's tarsi or antennae was recorded. Each of the 30 replications for each neem formulation was composed of five petri dishes. The two-by-two table test and Yates' corrected chi-square were used to detect treatment differences (Analytical Software 1998).

Neem-Based Formulation Application to Excised Squares. In a choice assay, two 7-mm-diameter debracted squares dipped in one of the neem formulations and two nontreated squares were placed, each in a randomly selected quadrant, in a 14-cm-diameter petri dish. One gravid female boll weevil was released into each petri dish and observed at 10-min intervals for the first 90 min, and then at 2.5, 3.5, and 4.5 h after the assay was initiated. The position of each boll weevil at each observation time was recorded as being on a neem-treated square, a nontreated square, or not on a square. Five separate petri dishes constituted each of 12 replications. After 24 h, feeding and oviposition damages to the squares were assessed (n = 60, each petri dish was a replicate). In the no-choice assay, conditions were the same except there were either two neem-treated or two nontreated squares (control) in each petri dish. Repeated measures analyses were run to assess the effects of treatment and time on the numbers of boll weevils on the squares in the choice and no-choice assays. The two-sample t-test and Yates' corrected chi-square test were used to detect treatment effects for the 24-h feeding and oviposition damage in the no-choice and the choice assays, respectively, and correlation analyses of dosages with the 24-h feeding and oviposition damages in the no-choice and the choice assays were run (Analytical Software 1998).

Choice Assay between All Four Neem-Based Formulations. Four 7-mm-diameter debracted cotton squares, each dipped in one of the four neem-based formulations, were placed equidistant from one another in a 14-cm-diameter petri dish. One gravid female boll weevil was released in each dish, n = 40. Numbers of egg and feeding punctures were recorded after 24 h. Data were analyzed using the Kruskal-Wallis one-way nonparametric analysis of variance (Analytical Software 1998).

Aging Neem in Sunlight. Forty-five cotton squares 5–7 mm in diameter on potted cotton plants were dipped in Agroneem (excess squares were removed). The cotton plants were set in a sunny outdoor location during May and June, 2002, at SARC when ambient daytime temperatures were 32.2–40.6°C. Fifteen of the squares were excised after 24 h, and each square was placed in a separate petri dish with a gravid boll weevil. Numbers of feeding and oviposition punctures on the square were recorded after 24 h. This process was repeated using squares aged on the whole plants for 48 and 72 h. Squares dipped in the Ecozin, and neem bitters solution, and nontreated controls were assayed in the same way. One-way analysis of variance (ANOVA) was used to detect differences between all of the treatments, and mean separation was accomplished with Tukey's honestly significant difference (HSD) (Analytical Software 1998).

Table 1. Mean numbers of boll weevils that first contacted excised 7-mm-diameter cotton squares dipped in neem-based formulations or nontreated controls

Assay[a]	Mean (±SE) no. first contact
Agroneem	2.0 ± 0.2
Control	3.0 ± 0.2
χ^2, *P*	2.27, 0.1316
Neemix	2.2 ± 0.2
Control	2.8 ± 0.2
χ^2, *P*	1.08, 0.2976
Ecozin	1.50 ± 0.20
Control	3.50 ± 0.20
χ^2, *P*	11.68, 0.0006
Bitters	0.8 ± 0.1
Control	4.2 ± 0.1
χ^2, *P*	37.69, <0.0001

[a] $n = 30$ replicates, one replicate = 5 petri dishes, 1 weevil per dish, Yate's corrected χ^2 test.

Results

No toxic effects of any of the neem formulations against the boll weevil adults were observed in this study.

First Contact with Treated or Nontreated Excised Squares. Gravid female boll weevils first contacted control squares 1.5-, 2.3-, 1.3-, and 5.3-fold more often than Agroneem-, Ecozin-, Neemix-, and bitters-treated squares, respectively. Only the Ecozin and bitters caused significant ($P \leq 0.05$) deterrence (Table 1).

Neem-Based Formulation Application to Excised Squares. Repeated measures analyses of the choice assays showed that female boll weevils were positioned on the nontreated squares (control 1) significantly more than on any of the neem-treated squares (Table 2). At 150 and 210 min, there was a significant increase of weevils on the treated squares. By 270 min, the mean numbers of boll weevils were not significantly different between the treated and control squares (Fig. 1A). The mean number of boll weevils that were not positioned on the treated squares (control 2, number on the control squares + the number not on any square) were significantly greater than on the Agroneem-treated squares throughout the assay. The mean number of weevils on controls 1 and 2 were significantly greater than the mean number on the Ecozin-treated squares at each sampling time (Fig. 1B). Weevils were positioned on the control 1 squares significantly more than on the Neemix-treated squares for the first 30 min and at the 150-min sampling time, but at the 210-min sampling time there were substantially fewer weevils than on the Neemix-treated squares (Fig. 1C). Mean numbers of weevils on the control 2 squares were consistently greater compared with the Neemix-treated squares. Mean numbers of weevils on the controls 1 and 2 were significantly greater than the mean numbers on the bitters-treated squares at each sampling time (Fig. 1D). Significant time effects were not detected in any of the four choice assays, but significant treatment by time interactions were found for comparisons between each neem treatment and their corresponding controls 1 and 2 (Table 2).

After 24 h in the choice assays, the mean numbers of egg punctures on the Ecozin- and the bitters-treated squares were significantly lower than their corresponding controls, but differences were not detected for Agroneem or Neemix (Table 3). The mean numbers of feeding punctures on the squares treated with Ecozin and bitters were significantly lower than their corresponding controls, but no differences were detected for Agroneem or Neemix (Table 3).

In the no-choice assay, repeated measures analyses showed significant treatment ($F = 291.07$; df = 4, 660; $P < 0.0001$) and time ($F = 11.29$; df = 11, 660; $P < 0.0001$) effects, and a treatment by time interaction ($F = 5.26$; df = 44, 660; $P < 0.0001$). For the first 90 min,

Table 2. Comparisons, using repeated measures analyses, of four formulations of neem extract for deterrent effects against boll weevils, choice assays

Choices[c]	Treatment effect[a]		Time effect[b]		Treatment*time interaction[b]	
	F	*P*	*F*	*P*	*F*	*P*
Agroneem						
Control 1	1742.98	<0.0001	0.01	1.000	13.50	<0.0001
Control 2	793.65	<0.0001	0.38	0.963	8.87	<0.0001
Ecozin						
Control 1	1276.90	<0.0001	0.00	1.000	9.81	<0.0001
Control 2	600.90	<0.0001	1.48	0.163	5.91	<0.0001
Neemix						
Control 1	297.99	<0.0001	0.00	1.000	4.22	<0.0001
Control 2	17.98	<0.0001	0.35	0.974	3.21	0.0004
Bitters						
Control 1	2120.95	<0.0001	0.20	0.997	5.62	<0.0001
Control 2	545.37	<0.0001	1.51	0.127	3.39	0.0002

[a] df = 1,264.

[b] df = 11,264.

[c] Two cotton squares of each choice were placed at random in a petri dish with one gravid female boll weevil; 5 dishes = 1 replicate, $n = 12$. *F* and *P* values are in the control rows because each of the controls is compared with its corresponding neem treatment; control 1, numbers of boll weevils on the nontreated squares and on no squares; control 2, numbers of boll weevils on the nontreated squares only.

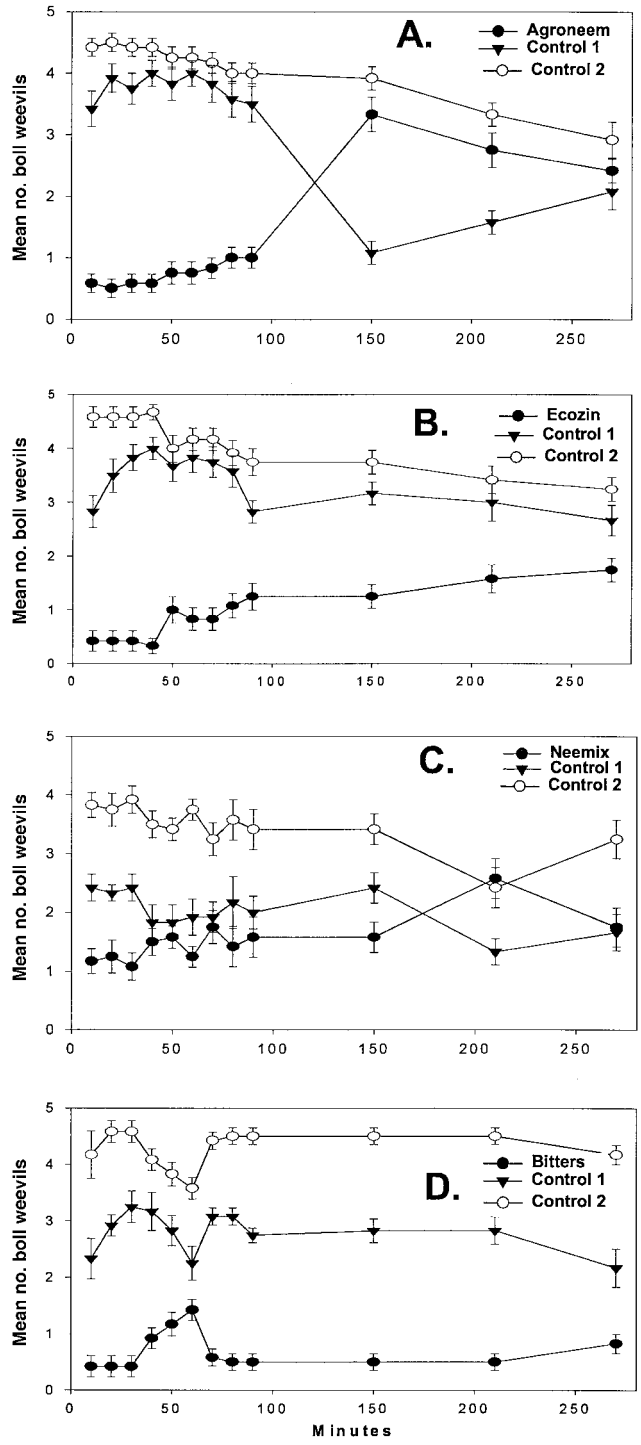

Fig. 1. Mean numbers (±SE) of gravid female boll weevils positioned on (A) Agroneem-, (B) Ecozin-, (C) Neemix-, and (D) bitters-treated cotton squares, each compared with control 1, numbers on nontreated squares; and control 2, numbers on nontreated squares + numbers not on any squares in petri dish assays, $n = 12$, one replication = 5 dishes. One weevil was exposed to two of the same neem treated squares and two control squares in each dish.

Table 3. Mean numbers of boll weevil feeding and oviposition punctures on cotton squares dipped in four neem extract formulations compared with nontreated controls after 24-h, choice assays

Choice[a]	Mean (±SE) no. feeding punctures	Mean (±SE) no. oviposition punctures
Ecozin	2.47 ± 0.36	2.03 ± 0.24
Control	3.55 ± 0.43	2.92 ± 0.30
χ^2, P	5.53, 0.0186	4.41, 0.036
Agroneem	2.89 ± 0.41	2.25 ± 0.24
Control	3.04 ± 0.39	2.92 ± 0.30
χ^2, P	2.14, 0.624	2.34, 0.1262
Neemix	3.02 ± 0.43	4.07 ± 0.48
Control	2.98 ± 0.41	5.00 ± 0.42
χ^2, P	0.00, 1.00	2.69, 0.1012
Bitters	2.03 ± 0.26	3.22 ± 0.32
Control	5.85 ± 0.72	5.93 ± 0.50
χ^2, P	57.81, <0.0001	24.12, <0.0001

[a] Two cotton squares of each choice (treated and nontreated control) were placed at random in a petri dish with one gravid female boll weevil; 1 dish = 1 replicate; n = 60; Yates corrected χ^2.

more weevils were observed on the control squares than on any of the treated squares (Fig. 2). Ecozin and bitters were more repellent than Agroneem and Neemix at 150 and 210 min. At 270 min, weevils were more numerous on Neemix-treated squares than the control, and differences between the other three neem treatments and the control were not detected.

After 24 h in the no-choice assay, significant treatment effects were detected for the mean number of egg punctures (F = 8.16; df = 4, 299; $P < 0.0001$) and the mean numbers of feeding punctures (F = 3.03; df = 4, 299; $P \leq 0.0180$). There were more ($P \leq 0.05$) egg punctures on the Neemix- and the bitters-treated squares than the Ecozin-treated squares (Table 4). No significant differences for feeding punctures were found between any of the neem treatments and the control, but feeding damage to Neemix-treated squares was greater ($P \leq 0.05$) than the bitters treatment (Table 4). No correlations ($P \geq 0.05$) were detected between azadirachtin content and oviposition or feeding deterrence.

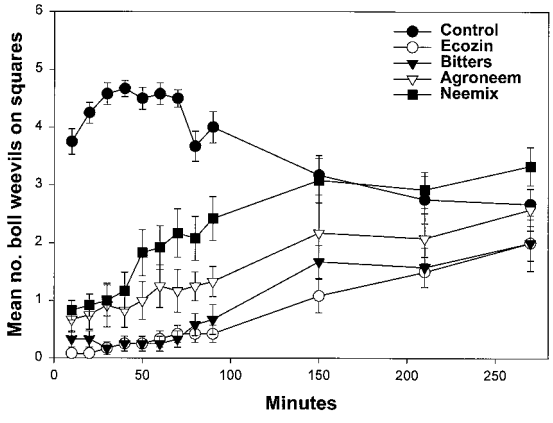

Fig. 2. Mean numbers (±SE) of gravid female boll weevils positioned on Agroneem-, Ecozin-, Neemix-, and bitters-treated, and control cotton squares in a petri dish no-choice assay, n = 12, one replication = 5 dishes. One weevil was exposed to two of the same neem treated squares or two control squares in each dish.

Table 4. Mean numbers of boll weevil feeding and oviposition punctures on cotton squares dipped in four neem extract formulations compared with nontreated controls after 24-h, no-choice assay

Choices[a]	Mean (±SE) no. feeding punctures	Mean (±SE) no. oviposition punctures
Agroneem	3.82 ± 0.33ab	1.60 ± 0.25bc
Ecozin	4.05 ± 0.30ab	1.28 ± 0.22c
Neemix	4.70 ± 0.29a	2.61 ± 0.31ab
Bitters	3.15 ± 0.42b	2.55 ± 0.31ab
Control	4.13 ± 0.24ab	1.68 ± 0.27bc

[a] Two cotton squares of each choice (treated and nontreated control) were placed at random in a petri dish with one gravid female boll weevil; 1 dish = 1 replicate; n = 60; one-way ANOVA, Tukey's mean separation ($P \leq 0.05$).

Choice Assay between All Four Neem-Based Formulations. No significant differences in the mean numbers of egg (Kruskel–Wallis statistic = 0.8256; df = 3, 159; P = 0.8433) or feeding (Kruskel-Wallis statistic = 3.589; df = 3, 159; P = 0.3094) punctures were found between the four treatments. Significant correlations were not detected between azadirachtin levels and oviposition and feeding deterrence.

Aging Neem in Sunlight. Mean numbers of egg punctures and feeding punctures on the control squares were not statistically different ($P \geq 0.05$) between the 24-, 48-, and 72-h assays (Fig. 3A and B). However, there was a significant overall treatment effect for egg punctures (F = 6.14; df = 14, 224; $P <$ 0.0001). After 24-h exposure to outdoor conditions, the Agroneem, Ecozin, Neemix, and bitters treatments had ≈70, 62, 65, and 82%, respectively, fewer egg punctures than the control, but there were no significant ($P \geq 0.05$) differences between the four neem treatments (Fig. 3A). No significant ($P \geq 0.05$) differences were detected between any of the treatments after 48 h of aging, and the mean numbers of weevils on each neem treatment were not significantly different ($P \geq 0.05$) from the mean numbers on the 24 h treatments (Fig. 3A). When the squares were exposed to outdoor conditions for 72 h, there were no significant differences ($P \geq 0.05$) between any of the treatments (Fig. 3A and B). At 72 h, mean numbers of egg punctures on the Agroneem, Neemix, and bitters treatments were 2.8, 2.6, and 4.5 times greater ($P \leq$ 0.05), respectively, than in the 24-h assay (Fig. 3A). There was also a significant overall treatment effect for feeding punctures (F = 6.66; df = 14, 224; $P < 0.0001$). Feeding damage after treated squares had been aged 24 h was significantly lower ($P \leq 0.05$) on the Agroneem, Ecozin, Neemix, and bitters treatments by ≈50, 46, 48, and 61%, respectively, than on the control, but there were no significant differences ($P \geq 0.05$) between the four neem treatments (Fig. 3B). No significant differences ($P \geq 0.05$) were detected between any of the treatments when treated squares were aged for 48 h (Fig. 3B). No significant differences ($P \geq 0.05$)

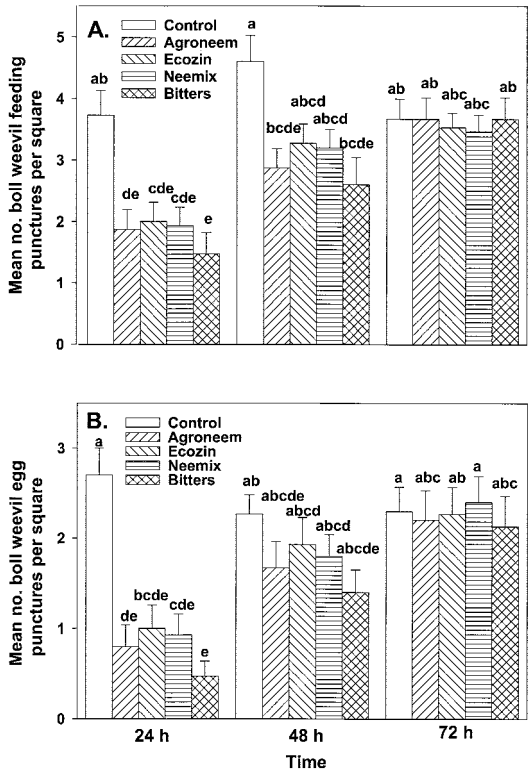

Fig. 3. Mean numbers (±SE) of (A) feeding and (B) egg punctures on two cotton squares ($n = 15$) that were aged outdoors on living plants for 24, 48, and 72 h after being treated with Agroneem, Ecozin, Neemix, or bitters neem-based formulations.

were detected between the 24- and 48-h ages for each of the neem treatments (Fig. 3B). The 72-h aged squares showed no differences ($P \geq 0.05$) between any of the treatments, but the Agroneem, Neemix, and bitters treatments had 1.96, 2.2, and 2.5 times more ($P \leq 0.05$) feeding punctures than the same treatments at 24 h.

Discussion

The "first contact" assay demonstrated that the Ecozin and bitters formulations repelled gravid female boll weevils as a result of volatiles, but this did not occur with Agroneem or Neemix. Schmutterer (1990) reported that 3% neem oil resulted in significantly fewer brown rice planthoppers, *Nilaparvata lugens* (Stål), landing on treated rice plants, which supports our findings that some neem formulations have olfactory effects. Our study showed that gravid female boll weevils are repelled from settling on squares treated with each of the neem-based formulations, but based on the choice comparisons against control one in the choice assays, the effects of Agroneem and Neemix did not last beyond three hours. Ecozin and bitters were repellent up to, and possibly beyond, 4.5 h. The no-choice assay demonstrated that Neemix was not repellent after the 90-min sampling time, and the repellency of the other three neem-based formulations gradually declined throughout the 4.5-h assay. Neem extracts have been reported as being repellent to a variety of insects (>400 species), including weevils (Jilani et al. 1988, Schmutterer 1990, Xie et al. 1995). Aerts and Mordue (1997) found that triterpenoids in neem extract, including azadirachtin, were repellent to *Spodoptera littoralis* (Boisdval) and to desert locust, *Schistocerca gregaria* (Forskål), nymphs. However, based on the azadirachtin content of the bitters, which was the lowest of the four neem-based formulations but showed a relatively high repellency, azadirachtin might not play the major role in the deterrence observed in our study. The treatment*time interactions resulted from opposite trends between each of the controls and the neem treatments, an expected outcome of some choice assays.

Feeding and oviposition punctures usually cause the square to abscise, resulting in crop loss (Cross 1973). The choice assay showed that after 24 h, Ecozin and bitters provided protection from feeding (30 and 65%, respectively) and oviposition (31 and 46%, respectively), but Agroneem and Neemix did not suppress injury from feeding or oviposition. In the no-choice assay, none of the treatments suppressed feeding or oviposition damage after 24 h. Azadirachtin is reported as being an antifeedant (Isman 1993, Mordue and Blackwell 1993; Liang et al. 2003) and an oviposition deterrent (Koul et al. 1990, Schmutterer 1990, Prabhaker et al. 1999) to a variety of insects, but this did not occur in our no-choice assay, except when the neem-treated squares formulations were exposed to outdoor conditions for 24 h after application and before weevils were introduced. Azadirachtin can be a phagostimulant to some insects (Simmonds and Blaney 1996) and in others it has no effects (Flint and Parks 1989, Schmutterer 1990), which seems to have occurred in the no-choice assay. Cotton squares with 24-h aged neem provided significant protection from feeding and oviposition damage during the 24 h in which gravid female boll weevils were present. Further aging of neem-treated squares resulted in declining deterrence until no more feeding or oviposition suppression was observed on the neem formulation-treated squares that had been aged for 72 h. Desensitization to the deterrent effects of azadirachtin is known to occur in some insects after repeated exposures (Bomford and Isman 1996). However, analogs and breakdown products of azadirachtin have been shown to be active against some insects (Barnby et al. 1989, Aerts and Mordue 1997, Mordue et al. 1997) and such breakdown could have occurred during the first 24 h in outdoor conditions because UV radiation rapidly reduces azadirachtin to component parts (Schmutterer 1990). Eventually, azadirachtin is degraded so that component parts are no longer active against insects (Koul et al. 1990, Schmutterer 1990). The observed absence of feeding and oviposition deterrence to neem formulation-treated squares that were not aged or exposed to sunlight might have been because azadirachtin did not break down in the same

way, or at the same rate, under laboratory conditions. This study further indicates that azadirachtin probably plays a role in causing deterrence, but other compounds in the neem-based formulations likely had as much, or greater, influence on deterrence of gravid female boll weevil feeding and oviposition. This conclusion is supported by the choice assay in which squares treated with the four neem-based formulations in the same petri dish were not significantly affected by any one of the treatments over the others.

Neem has been shown to reduce populations of silverleaf whitefly, *Bemisia argentifolii* (Bellows & Perring); sweetpotato whitefly, *Bemisia tabaci* (Gennadius); and cotton aphids, *Aphis gossypii* Glover, in cotton (Coudriet et al. 1985, Flint and Parks 1989, Butler et al. 1991, Akey and Henneberry 1999), but a commercial neem-based formulation with 160 ppm azadirachtin did not consistently reduce populations of flower thrips, *Frankliniella* spp.; cotton leaf perforator, *Bucculatrix thurberiella* Busck; and spider mites, *Tetranychus* spp., in cotton plots (Flint and Parks 1989). Liang et al. (2003) found that although Agroneem, Ecozin, and Neemix did not reduce oviposition by the diamondback moth, *Plutella xylostella* L., all three formulations had significant antifeedant effects on the larvae. The neem formulations assessed in our study do not seem to be suitable for commercial use against boll weevils because their deterrent effects are short-lived in outdoor conditions (<48 h). Given the large populations of boll weevils that can occur in areas where boll weevil eradication has not occurred, (e.g., the Lower Rio Grande Valley of Texas and Mexico) (Showler 2003), the neem-based insecticides evaluated in this study would need to be applied at least three times weekly, which would be impractical and likely not cost-effective. Although it seems that the neem-based formulations are inadequate for commercial adoption against boll weevils, further research on the other components of neem formulations that have biological activity against insects might provide means of managing boll weevil populations, particularly as increasing documentation of the negative environmental and health impacts of synthetic toxic insecticides and increasingly stringent government regulation of pesticides has renewed interest in botanical pest management products (Ascher 1993).

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